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Award Number: W81XWH-12-1-0337

TITLE: Molecular Innovations Toward Theranostics of Aggressive Prostate Cancer

PRINCIPAL INVESTIGATOR: Hsieh, Jer-Tsong

CONTRACTING ORGANIZATION: University of Texas Southwestern Medical Center
Dallas, TX 75390

REPORT DATE: September 2013

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
September 2013	Annual	01 September 2012-31 August 2013

4. TITLE AND SUBTITLE
Molecular Innovations Toward Theranostics of Aggressive Prostate

5a. CONTRACT NUMBER
W81XWH-12-1-0337

5b. GRANT NUMBER
W81XWH-12-1-0337

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Jer-Tsong Hsieh

E-Mail: jt.hsieh@utsouthwestern.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of Texas Southwestern Medical Center
5323 Harry Hines Blvd.,
Dallas, TX 75390

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Conventional chemotherapy with cell killing en mass often targets mitotic cells with less specificity, which likely leads to undesirable side effect. Knowing specific molecular defects in cancer cells has led to discover new chemotherapeutic agents. Thus, combined agents targeting different defected pathways in cancer cells have a better chance to eradicate tumor completely. Thus, to achieve a cure, a comprehensive targeting strategy needs to be implemented. In addition, improved methods for monitoring drug delivery and tumor response in a nearly real-time manner should offer a safe and effective treatment. This project carried out by a team of chemist, radiologist, and molecular tumor biologist is to develop a novel drug delivery system with new small molecular therapeutic agents assisted with new imaging probe is expect to bring a new frontier for prostate cancer management. Our objective is to develop dendrimer-based theranostic agent with prostate cancer specificity and positron emission tomography imaging capability that can prevent the early onset of PCa metastasis or delay the progression of metastasis. The mission of my project is to design small peptide derived from tumor suppressor DAB2 family as therapeutic agent and examine its biology activities.

15. SUBJECT TERMS none provided

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
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INTRODUCTION

This project combines the recent advances in PCa research from three different laboratories to develop a new molecular medicine. The goal of this project is to construct dendrimer nanoconjuate containing a prostate specific cell permeation peptide, peptide therapeutic(s) and bifunctional chelator for PET imaging. Dr. Simanek's laboratory will make dendrimers that bear functional handles for conjugation with imaging agents (from Dr. Sun's laboratory) and proline-rich peptide as a therapeutic agent (from my laboratory).

Research over the last few decades has provided detailed mechanistic insight into the different steps of metastasis. Recent insights have suggested yet another step, to be added at the beginning of the cascade: the creation of a "pre-metastatic niche" at the target site before the first tumor cells dissociate from bulk tumor. It appears that epithelial-to-mesenchymal transition (EMT) is a key mechanism to facilitate cancer cells to form the pre-metastatic niche (1, 2). From our previous publications (3-5), my laboratory identified the factors critical in EMT. Based on structural-functional analysis of these factors, we are formulating a new therapeutic strategy to intervene PCa invasion or metastases. Specifically, we will synthesize small peptide derived from proline-rich domain (i.e., the functional domain) of these factors (3, 4, 6) to examine their therapeutic efficacies.

BODY

During the first year my laboratory has identified the most potent peptide from several synthetic peptides as a candidate. Currently, the progress of selecting therapeutic peptide is on schedule, I expect that we can complete the characterization in the next 6 months ahead of proposed schedule.

Aim 2: To select potent compounds with screening systems based on specific mechanism(s) of action.

Task 3 (Months 1– 24) Selection of therapeutic peptides.

We are planning to use a panel of 6 different prostate cell lines without DAB2IP expression, which represent a full spectrum of PCa progression including tumorigenic prostate epithelia, metastatic PCa.

To select the most potent peptide, Dr. Simanek has synthesized 9 different small peptides (InpP10, InpR11, R11P8, P10R9, R11AAL, CS-IV-78, CS-IV-81, R11P10, R11PPL) based on the consideration for chemical conjugation with dendrimer nanoparticle; R11 and P10 were used as a negative control. In selecting the most potent peptide, we have used 3 different PCa cell lines (LAPC4, PC-3 and C4-2); C4-2 without endogenous DAB2IP expression and knockdown DAB2IP expression in both LAPC4 and PC-3 cell lines. Also, these 3 cell lines represent many features of metastatic prostate cancer, for example, both LAPC4 and C4-2 are androgen receptor-positive cells from lymph node metastases and PC-3 is androgen receptor-negative cell from bone metastases. In this experiment, we determined Akt and Src (cell survival and proliferation pathway)

activation based on their phosphorylation level, and E-cadherin and vimentin represent EMT status. As shown in Fig. 1, CS-IV-81, R11P10, R11PPL exhibited significant inhibition of cell survival, proliferation and EMT. Both R11P10 and R11PPL are prototype peptides but CS-IV-81 is chemical modified R11P10 suitable for chemical conjugation with nanoparticle.

We are in the process of performing these experiments: (1) to determine EMT phenotypes (morphology, cell migration/invasion) *in vitro* and (2) tumor invasiveness and metastases using orthotopic model.

KEY RESEARCH ACCOMPLISHMENTS

- Design different small peptides based on the need for conjugating with dendrimer nanoparticle.
- Characterize and select the most potent small peptide from several metastatic prostate cancer models.
- Publish 2 peer-reviewed papers: one is review paper based on current progress of dendrimer nanoparticle construction and the other is to report new design of a multivalent PET imaging probe.

REPORTABLE OUTCOMES

- Lo, S., Kumar, A., Hsieh, J.T., Sun, X. (2013) Dendrimer nanoscaffolds for potential theranostics in prostate cancer. Mol. Pharm., 10:793-812. (http://www.ncbi.nlm.nih.gov/pubmed/23294202)

- Hao, G., Kumar, A., Dobin, T., Oz, O., Hsieh, J.T., Sun, X. (2013) A multivalent approach of imaging probe design to overcome an endogenous anion binding competition for noninvasive assessment of prostate specific membrane antigen. Mol. Pharm., 10:2975-2985. (http://www.ncbi.nlm.nih.gov/pubmed/23768233)

CONCLUSION

In the first year, we have designed different chemical modification of small peptide and characterized their *in vitro* biologic activities using several metastatic prostate cancer cell lines. Our data have shown the better activity of chemical modified peptide than prototype peptide, which led us to unveil new candidate peptide for future assembly with dendrimer.

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Appendices

Figure 1 Characterization of biologic activities of chemical modified small peptides in metastatic prostate cancer cell lines. Each cell line was incubated with peptide for 30 minutes then removed peptide with multiple washing. Cell was harvested 48 hours after incubation and subjected to western blot analysis with different markers representing cell survival, proliferation and EMT.